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Angiogenesis in Follicular Tumors of the Thyroid

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Background: Experimental evidence suggests that tumor growth beyond a certain size and tumor ability to metastasize depend on the degree to which the tumor can stimulate an angiogenic response.

Methods: Fifteen thyroid specimens of microinvasive follicular carcinoma were examined for vascularization by immunohistochemical stain with antifactor VIII antibodies and compared with an equal number of follicular adenomas.

Results: Pleomorphic areas in the histological specimens of follicular carcinomas had a higher rate of vascularization as did areas of tumor adjacent to or penetrating the capsule. These features were not noted in follicular adenomas.

Conclusions: Our findings indicate that vascularity may play a role in extracapsular extension and tumor aggression in follicular thyroid carcinomas.

KEY WORDS: **angiogenesis, thyroid, follicular tumors**

INTRODUCTION

There is increasing evidence to support the observation that tumor growth and, especially, tumor ability to metastasize, are dependent on blood vessel formation, or neovascularization. The term angiogenesis was first coined in 1935 to describe new blood vessel formation in the placenta [1]. Findings in histological sections of tumors with specially stained capillaries strengthened the idea that tumor growth is linked to capillary formation [2]. For two decades there was disagreement as to whether tumors were supplied by existing vessels or by neovascularization.

Not until 1968, when tumors were shown to secrete factors inducing growth of new capillaries, was this question settled. The first isolation of an angiogenic factor from tumor was reported in the early 1970s [3], and during the next two decades several more were demonstrated. One of the factors that was strongly associated with angiogenesis is vascular endothelial growth factor (VEGF), a strong mitogen causing proliferation of the vascular endothelium. Its inhibition has also been shown to result in reduction of tumor growth [4].

The present study was undertaken to examine the place of neovascularization in the tumorigenic process of follicular carcinomas originating in the thyroid gland. It attempts to determine whether angiogenesis plays a role in extracapsular spread and its precise association with distant metastases. Anti-Von Willebrand factor was used, which stains vascular endothelium and facilitates the detection of blood vessels.

MATERIALS AND METHODS

Paraffin-embedded samples of follicular thyroid tumors from 30 patients were obtained from the archives of the Department of Pathology, Beilinson Medical Center. Of these, 15 were microinvasive follicular carcinomas and 15 follicular adenomas. Two 5 μm-thick sections from each block were cut. The sections were deparaffinized in xylene and rehydrated with graded concentrations

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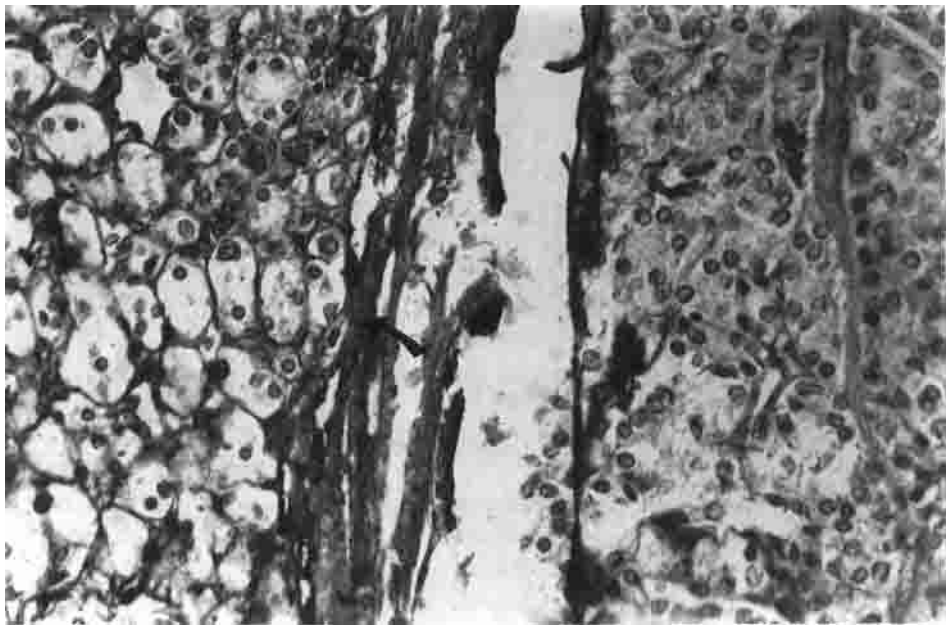

Fig. 1. Follicular carcinoma showing increased vascularity in area of capsule infiltration (*arrow*). (Factor 8 immunostain, ×250.)

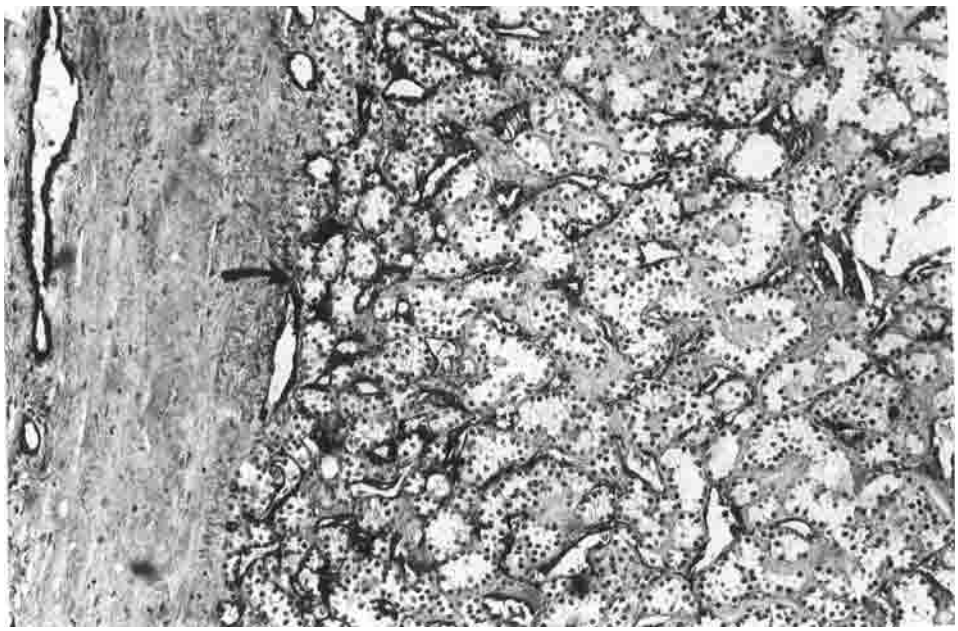

Fig. 2. Follicular carcinoma showing increased vascularity in area adjacent to the capsule (*arrow*) and decreased vascularity distant from the capsule. (Factor 8 immunostain, ×100.)

of alcohol. Sections were incubated with Von Willebrand factor 1:100 for 1 hr at room temperature in a moist chamber. The sections then were washed in phosphate-buffered saline (PBS) for 15 min and incubated with goat anti-Von Willebrand factor at 1:100 for 1 hr and again at room temperature. These sections were washed again in PBS and incubated with rabbit antigoat IgG 1:10 for 30 min. Sections were washed in PBS and incubated with goat peroxidase antiperoxidase 1:50 for 30 min. After three washes in PBS, sections were immersed in 0.05% diaminobenzidine (DAB) in 0.05 nm Tris–HCl buffer pH 7.6 containing 0.01% H_2O_2 for 2 min. Sections were washed in running water, counterstained in Carazzi's hematoxylin, dehydrated, cleared, and mounted with paramount.

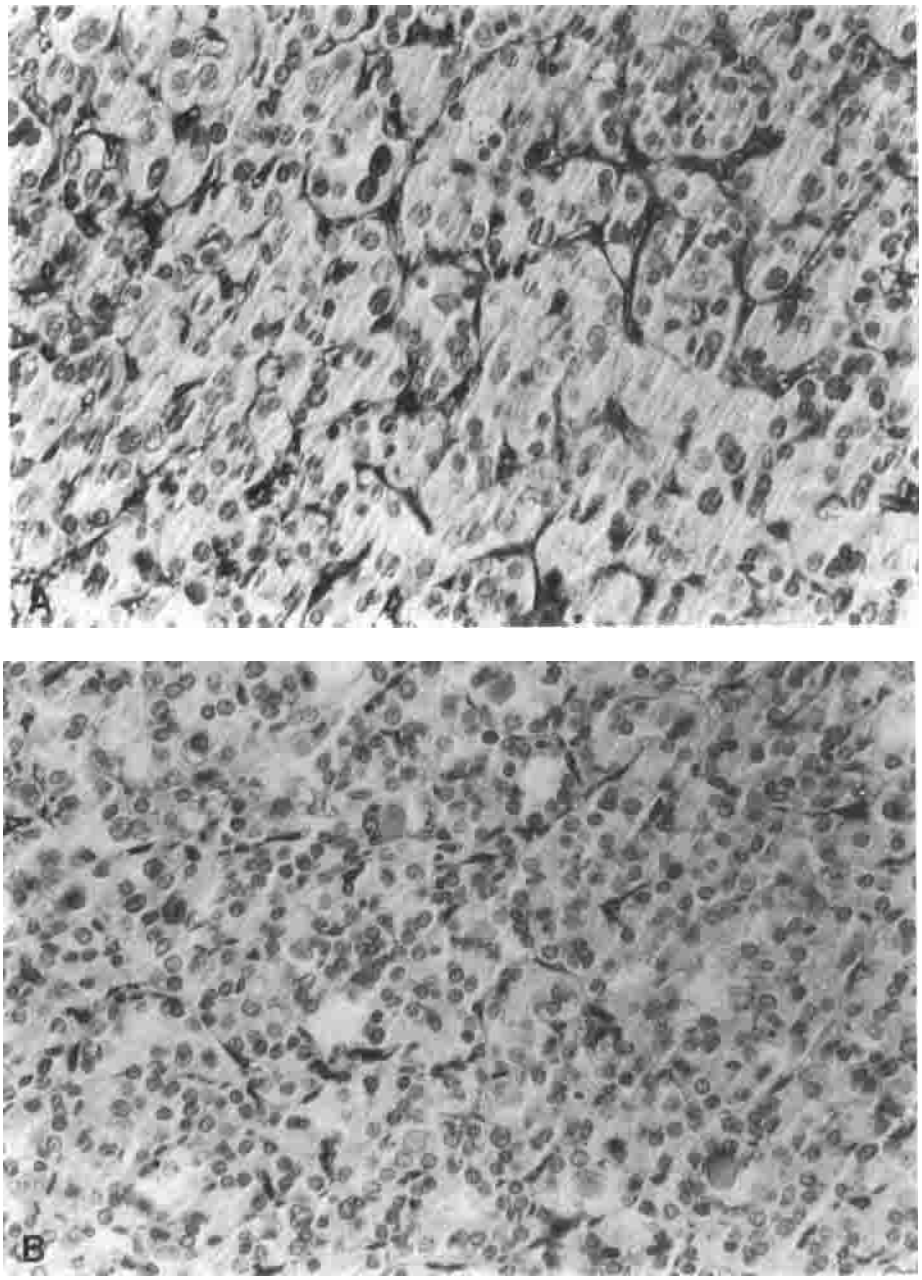

Fig. 3. **A:** Follicular carcinoma showing marked pleomorphism and increased vascularity. (Factor 8 immunostain, ×250.) **B:** Follicular carcinoma showing minimal pleomorphism and low vascularity. (Factor 8 immunostain, ×250.)

RESULTS

A marginal difference was noted in the degree of vascularity between the follicular thyroid adenomas and the follicular thyroid carcinomas; both types were relatively vascular. Eight of the 15 adenomas examined were more vascular than the other seven. The same was true for the follicular carcinomas. However, a major difference in vascularity was noted among different areas within the follicular carcinomas.

Areas of follicular carcinomas adjacent to and, especially, infiltrating the capsule showed significantly increased vascularity (Fig. 1), with a ratio of one blood vessel to two tumor cells (Fig. 2, arrow). Tumor areas distant from the capsule had a ratio of only one vessel to 10 tumor cells on average (Fig. 2).

Areas of adenomas adjacent to the capsule did not have prominent vascularity, in contrast to the carcinomas. Their ratio of blood vessels to tumor cells was uniformly around 1:10. Solid areas with marked pleomorphism within the follicular carcinomas, suggesting a higher degree of ma-

lignancy, again showed a higher ratio of vascularity (Fig. 3A) than areas with no marked pleomorphism or solid formation (Fig. 3B). A 1:2 blood vessel tumor cell ratio was noticed in the pleomorphic sites and solid areas (Fig. 3A), whereas a much lower ratio 1:10 was observed in other zones (Fig. 3B).

DISCUSSION

There is to date no accurate, reliable method by which benign thyroid nodules can be differentiated from malignant tumors. As a result, many patients with thyroid nodules undergo unnecessary surgery. Depending on the series quoted, 60–80% of thyroid operations performed because of suspected cancer could be prevented if a reliable marker for thyroid malignancy were available. The large number of unjustified operations places a considerable burden on health care systems [5].

During the 1950s, progress in two different directions eased this problem to some degree. New imaging techniques, such as radioactive scans and ultrasound were able to screen out those patients with thyroid nodules at a higher risk of malignancy [5,6]. The introduction of fine-needle aspiration biopsy led to a further decrease in the number of unnecessary procedures [5,7–9]. However, patients with follicular tumors did not benefit from these innovations and, when a follicular tumor is suspected, the patient will almost always end up in the operating room [10].

Vascularity has recently been shown to play a decisive role in tumor ability to grow and expand beyond a certain size and to metastasize. In the present study we examined vascularity as a potential marker for follicular thyroid carcinomas. Such a marker would not only promote our understanding of the malignant process but would also assist in the selection of patients for surgery. Anti-Von Willebrand factor was used because it stains vascular endothelium, making small vessels easily detectable. Factor 8 is therefore accepted as a marker for vascularity.

In comparing the degree of vascularity between follicular adenomas and adenocarcinomas, no significant differences were found. However, a definite difference in vascularization was noted in different areas within the follicular carcinomas. We showed that the more malignant appearing areas, as indicated by pleomorphism and solidity, had a higher rate of vascularization. Areas of tumor adjacent to or penetrating the capsule were also characterized by high vascularity. Thus, although vascularity did not seem to be a distinguishing feature of follicular carcinoma, the higher vascularity in the more malignant areas of the tumor suggests that vascularity may indeed play a role in tumor aggression. The presence of high vascularity in the pericapsular area also suggests that vascularity may be important in tumor potential for extracapsular extension and expansion. Further studies investigating the expression of vascular endothelial growth factors in follicular thyroid tumors are scheduled to follow.

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